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ORIGINAL ARTICLE

# Hematology of Wild Caught *Hoplobatrachus rugulosus* in Northern Thailand

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**Abstract** The rice field frog *Hoplobatrachus rugulosus* is an important anuran species found in wetlands throughout Thailand. At present, the hematological parameters of wild populations are unknown. Therefore, hematological and morphological characteristics of peripheral blood cells of wild-caught *H. rugulosus* were examined. Thirty-three adult frogs (17 male and 16 female frogs) were collected from a natural population in Nan Province, northern Thailand during the wet season of 2014. Blood samples were analyzed by packed cell volume (PCV) and blood cell counts from hemocytometer and Giemsa-stained blood smears. The mean PCV of male frogs (30.70% ± 6.07%) was significantly higher than that of the female frogs (25.09% ± 4.85%). The mean number of lymphocytes and neutrophils also showed significant sex-related differences. Moreover, the morphometric analysis of blood cells revealed dimensions as follows: erythrocytes (17.96 ± 1.44 μm length × 11.50 ± 1.09 μm width), immature erythrocytes (14.91 ± 2.20 μm diameter), thrombocytes (13.93 ± 3.14 μm length × 7.05 ± 1.31 μm width), lymphocytes (11.01 ± 2.69 μm diameter), monocytes (12.04 ± 2.40 μm diameter), neutrophils (12.58 ± 2.08 μm diameter), basophils (13.60 ± 2.17 μm diameter) and eosinophils (12.33 ± 2.95 μm diameter). Overall, the hematological parameters obtained in this study could be regarded as the first report and a crucial baseline data of wild *H. rugulosus* in Thailand that can be used for monitoring the health status of this anuran.

**Keywords** Rice field frog, erythrocyte, leukocyte, packed cell volume, morphometry, Nan Province

## 1. Introduction

Monitoring the health of wildlife is very important for their conservation and management. Among general health assessment approaches, hematological analyses are viewed as reliable methods to determine the health status in mammals and other vertebrates. Changes in some hematological parameters compared with the reference values may be used as evidence of physiological disturbances, such as xenobiotic exposure, and diseases or stress, in vertebrates (Bloom and Brandt, 2008; Davis *et al.*, 2008). Among the hematological parameters of peripheral blood that have been frequently used to assess the health status of animals are the packed cell volume (PCV) or hematocrit, erythrocyte count, leukocyte count and differential leukocyte count. Data on the hematological parameters of human and domestic mammals are well documented, but remain limited for amphibians and reptiles. With respect to amphibians the use of hematological parameters for health assessment has been reported in some species, such as the common toad (*Bufo arenarum*) in agricultural areas in Argentina (Cabagna *et al.*, 2005), the marsh frog (*Rana ridibunda*) in an industrial area in Bulgaria (Zhelev *et al.*, 2006), the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in a pesticide contaminated river in southern Indiana, USA (Burgmeier *et al.*, 2011) and the northern leopard frog (*Lithobates pipiens*) in pesticide contaminated areas in Canada (Shutler and Marcogliese, 2011). However, reference data on the hematological parameters of wild populations of frog species is scarce compared with the diversity of animals in this taxonomic group, even though it is crucial for their health assessment.

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The rice field frog (*Hoplobatrachus rugulosus*, Wiegmann, 1835) is an anuran amphibian in the family Dicroglossidae with a widespread distribution from central China and Myanmar to Thailand and peninsular Malaysia, where they are commonly found in wetlands and paddy fields (Diesmos *et al.*, 2004). Even though their natural habitats in Thailand have been disturbed and are very limited, natural populations can still be found in Nan Province, northern Thailand, and lowland sites in other provinces from central to southern Thailand (Pansook *et al.*, 2012). This frog is economically important in Thailand because it is used as food by the local people. It also has the potential to be used as a model for research in many fields, such as physiology, reproductive biology and ecotoxicology. At present, wild populations of *H. rugulosus* are of concern due to human-mediated over harvesting, habitat disturbances, stress and diseases, but the health status of these natural populations is unknown.

Until now, the hematological parameters in the natural populations of *H. rugulosus* are still undocumented. Only the hematological aspects of a closely related species, the common Indian frog (*Rana tigrina*) have been reported (Singh, 1977a, 1977b, 1978). Therefore, the purpose of this study was to identify the morphological characteristics of different peripheral blood cells and to determine the hematological parameters of the peripheral blood in *H. rugulosus*. Data from this study would then form the fundamental basis for monitoring of the health status of this natural anuran population.

## 2. Materials and Methods

**2.1 Frog collection and blood sampling** Adult *H. rugulosus* were collected from organic rice fields (UTM 0686779 2047187, zone line 47Q) with no history of herbicide usage for more than 10 years in Wiang Sa District, Nan Province, northern Thailand during the rainy season (July to August 2014). Thirty-three adult frogs (17 males, 16 females) were collected by the visual encounter survey method (Crump and Scott, 1994) from this natural habitat. Blood samples (0.5 mL per 100 g body weight) were collected from the frogs by cardiac puncture (Heatley and Johnson, 2009) under cold anesthesia using 25-gauge needle with heparinized tuberculin syringes and transferred to microcentrifuge tubes. The samples were stored in ice bucket during an hour of PCV, hemocytometer counting, and blood smear processes.

**2.2 Hematology** The PCV was determined after the blood sample had been transferred to a microcapillary tube and centrifuged at 8,700×g for 10 min. Erythrocyte and leukocyte counts were determined manually using a hemocytometer (Tharp and Woodman, 2002) after the blood sample was diluted with Natt and Herrick's solution (Natt and Herrick, 1951). Blood smears were prepared on glass slides immediately and fixed with absolute methanol. Giemsa staining (Bain and Lewis, 2012) was used to study the frog blood cell morphology and determination of the hematological parameters using light microscopy at 40× objective lens. On blood smear slide of each frog, a total of 200 leukocytes were counted for the differential leukocyte count. Immature and mature erythrocytes were counted in 10 fields with an aid of grid ocular micrometer and the percentage of immature erythrocyte was calculated (Briggs and Bain, 2012). For morphometric study of blood cell size, blood smears from 10 frogs (5 frogs per sex) were randomly selected out of 33 frogs. On each blood smear slide of a frog, the length and width of 30 randomly selected mature erythrocytes and their nuclei, and 30 randomly selected thrombocytes were measured using the Image-Pro Plus (ver. 6.00 software) from their digital images taken with a digital camera (Cannon EOS 550D). Selection of the areas from good spread blood film with no overlap of the cells and the cell number (30 cells) used in these measurements were based on a previous study by Kuramoto (1981). Likewise, the diameters of 30 randomly selected cells for each of lymphocytes, monocytes, neutrophils, basophils, eosinophils and immature erythrocytes, were also measured in the same manner. The mean value of each morphometric parameter in each frog was calculated from 30 cells, and the grand mean from 5 frogs was finally calculated. The erythrocyte cell and nuclear areas (EA and NA, respectively) were calculated according to the formula of the area of an elliptical shape (length × width × $\pi$/4), and the nucleocytoplasmic ratio (NA/EA) was calculated (Arikan and Cicek, 2010). In the same way the immature erythrocyte cell and nuclear areas (IEA and NIEA, respectively) were calculated according to the formula of the area of a circular shape [$\pi \times (\text{diameter}/2)^2$], and used to derive the nucleocytoplasmic ratio (NIEA/IEA).

**2.3 Statistical analysis** All hematological parameters of each sex were summarized as the mean, standard deviation (SD) and range. Statistical analyses were performed according to Zar (1998) using the SigmaPlot (ver. 11.00) statistical software. If the distribution of data was not significantly different from a normal distribution (Kolmogorov-Smirnov test, $P > 0.05$), the significance of differences in the means were compared using the Student's *t*-test otherwise they were compared using the

Mann-Whitney *U* test. Significance was accepted at the $P \le 0.05$ level.

## 3. Results

The hematological parameters of the peripheral blood of *H. rugulosus* are summarized in Table 1, where the mean erythrocyte count (derived from hemocytometer counting) of male frogs was numerically slightly (1.08-fold) higher but not significantly different from that of female frogs ($t = 0.38$, $df = 31$, $P = 0.71$). Likewise, the leukocyte count was numerically (1.25-fold) but not significantly higher in male frogs than in female frogs (Mann-Whitney *U* test, $T = 261.5$, $P = 0.71$). However, a significant difference in the mean PCV between the sexes was found, being 1.22-fold higher in male than in female frogs ($t = 2.84$, $df = 31$, $P = 0.01$).

Hematological examination of the peripheral blood smear slides revealed sex-related differences in some leukocyte parameters (Table 1), where the mean values of differential lymphocyte count of male frogs is significantly higher than that of the female frogs (1.1-fold, $t = 2.74$, $df = 31$, $P = 0.01$). Mean values of differential neutrophil count was significantly higher in male frogs than in female frogs (2.0-fold, $t = 2.12$, $df = 31$, $P = 0.04$).

Based on their morphological characteristics under light microscopy, the peripheral blood cells of *H. rugulosus* were classified into erythrocytes, immature erythrocytes, thrombocytes and leukocytes. The cytomorphological analyses revealed no significant differences between the sexes in the size of each cell type, allowing an average size of both sexes combined to be evaluated (Tables 2 and 3). Mature erythrocytes were large elliptically shaped cells with a centrally located elliptical shaped nucleus with dense basophilic chromatin. The nuclear membrane was very prominent under light microscopy and the cytoplasm stained light blue to colorless (Figure 1A). Immature (polychromatic) erythrocytes were found in the circulating blood of this frog species. They were spherical with a centrally located round nucleus that was larger and less basophilic staining than that of mature erythrocytes, while the cytoplasm

**Table 1** Blood cell counts and packed cell volume (PCV) of *H. rugulosus* collected from Nan Province, Thailand during July to August 2014.

| Parameters | Sex[a] | Mean | SD | Range |
|---|---|---|---|---|
| Erythrocyte ($\times 10^6$ cells/µL) | Male | 0.64 | 3.98 | 1.85–16.65 |
| | Female | 0.60 | 2.81 | 1.80–10.40 |
| | Both sexes | 0.62 | 3.42 | 1.80–16.65 |
| Leukocyte ($\times 10^3$ cells/µL) | Male | 5.31 | 3.74 | 1.13–13.00 |
| | Female | 4.26 | 1.74 | 1.68–6.75 |
| | Both sexes | 4.80 | 2.95 | 1.13–13.00 |
| Immature erythrocyte* (%) | Male | 5.46 | 1.59 | 3.00–8.83 |
| | Female | 8.19 | 2.15 | 4.16–12.50 |
| | Both sexes | 6.78 | 2.31 | 3.00–12.50 |
| Differential lymphocyte* (%) | Male | 57.49 | 6.56 | 45.00–69.00 |
| | Female | 51.66 | 5.57 | 42.50–62.50 |
| | Both sexes | 54.66 | 6.69 | 42.50–69.00 |
| Differential monocyte (%) | Male | 30.88 | 7.69 | 20.00–46.00 |
| | Female | 36.09 | 7.75 | 21.50–49.50 |
| | Both sexes | 33.41 | 8.05 | 20.00–49.50 |
| Differential neutrophil* (%) | Male | 1.82 | 1.36 | 0.00–4.15 |
| | Female | 0.92 | 1.04 | 0.00–3.59 |
| | Both sexes | 1.38 | 1.28 | 0.00–4.15 |
| Differential basophil (%) | Male | 0.77 | 0.72 | 0.00–2.04 |
| | Female | 0.41 | 0.42 | 0.00–1.03 |
| | Both sexes | 0.60 | 0.61 | 0.00–2.04 |
| Differential eosinophil (%) | Male | 9.05 | 2.55 | 3.52–13.57 |
| | Female | 10.92 | 4.16 | 5.58–20.50 |
| | Both sexes | 9.95 | 3.50 | 3.52–20.50 |
| PCV* (%) | Male | 30.70 | 6.07 | 20.50–39.00 |
| | Female | 25.09 | 4.85 | 11.00–32.00 |
| | Both sexes | 27.99 | 6.13 | 11.00–39.00 |

[a] Data are derived from 17 males, 16 females or both (33 males and females) frogs.

* Significant difference of mean parameter values between males and females, Student's *t*-test ($P \le 0.05$).

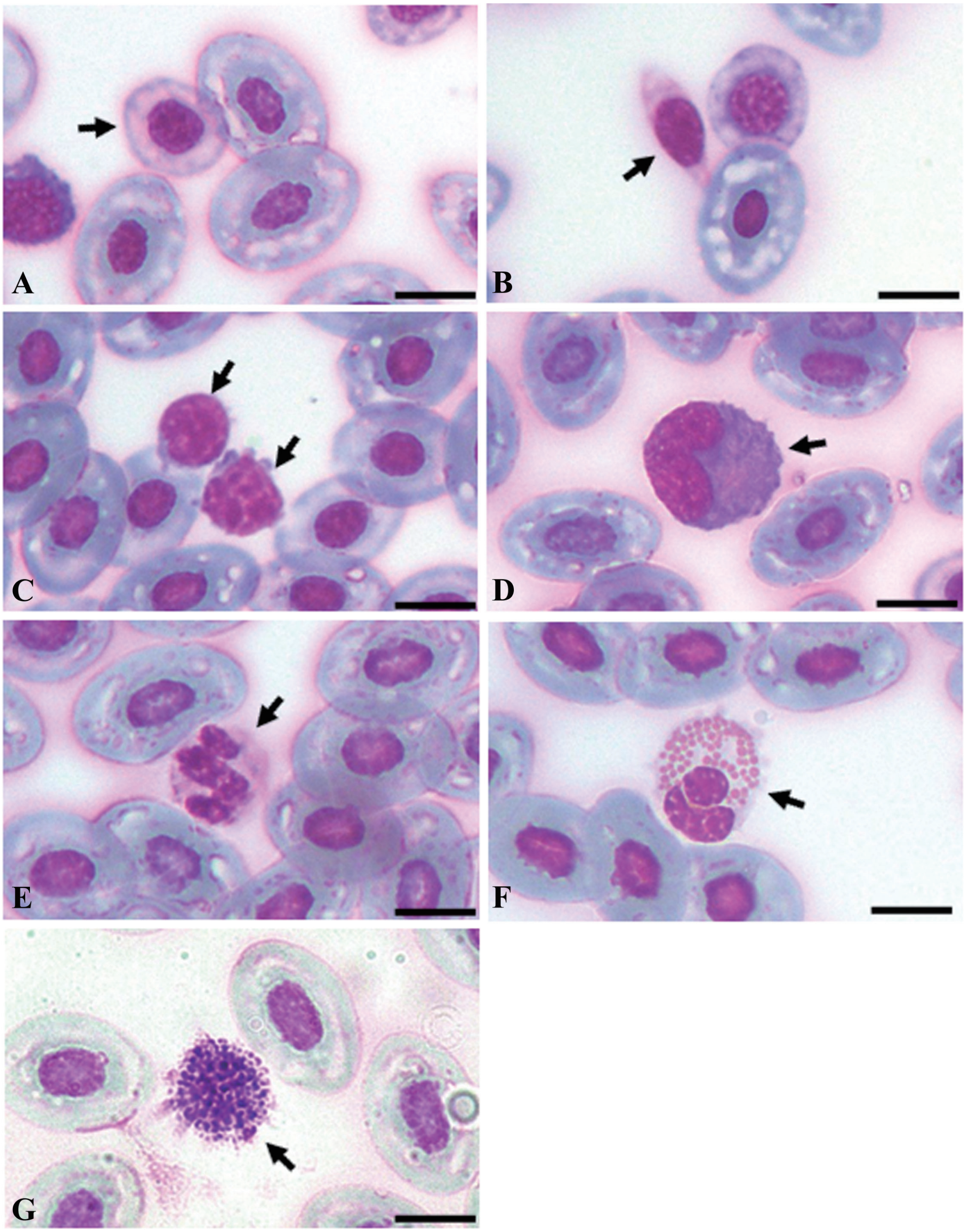


**Figure 1** Light micrographs showing the different types of blood cells in *H. rugulosus*. Erythrocytes were classified as (A) mature and immature (arrows) erythrocytes. (B) Thrombocytes. Leukocytes were classified as (C) lymphocytes (arrow), (D) monocytes (arrows), (E) neutrophils (arrow), (F) eosinophils (arrow), (G) basophils (arrow). Bar = 10 µm; Giemsa stain.

stained light blue or colorless (Figure 1A). Comparison of the nucleocytoplasmic ratios (Table 2) showed that the immature erythrocytes had a significantly higher (1.69-fold) nucleocytoplasmic ratio than that of the mature erythrocytes.

Thrombocytes were round to elliptical-shaped cells of a smaller size (1.29- and 1.93-fold in length and width, respectively) than mature erythrocytes and contained an

**Table 2** Morphometric data of mature and immature erythrocytes and thrombocytes of *H. rugulosus* collected from Nan Province, Thailand during July to August 2014.

| Characters | Sex[a] | Mean | SD | Range |
|---|---|---|---|---|
| Erythrocyte length (μm) | Male | 17.73 | 1.45 | 14.64–23.22 |
| | Female | 18.18 | 1.39 | 15.31–22.47 |
| | Both sexes | 17.96 | 1.44 | 14.64–23.22 |
| Erythrocyte width (μm) | Male | 11.17 | 0.99 | 8.72–14.40 |
| | Female | 11.83 | 1.08 | 9.25–15.89 |
| | Both sexes | 11.50 | 1.09 | 8.72–15.89 |
| Erythrocyte area (EA; μm$^2$) | Male | 155.32 | 16.80 | 117.07–213.79 |
| | Female | 169.41 | 24.49 | 117.78–267.11 |
| | Both sexes | 162.36 | 22.12 | 117.07–267.11 |
| Erythrocyte nuclear length (μm) | Male | 6.95 | 1.18 | 4.84–10.51 |
| | Female | 6.80 | 1.08 | 4.20–9.78 |
| | Both sexes | 6.87 | 1.14 | 4.20–10.51 |
| Erythrocyte nuclear width (μm) | Male | 4.69 | 1.15 | 2.34–8.36 |
| | Female | 4.55 | 0.62 | 3.06–6.48 |
| | Both sexes | 4.62 | 0.93 | 2.34–8.36 |
| Erythrocyte nuclear area (NA; μm$^2$) | Male | 26.13 | 10.40 | 12.15–64.95 |
| | Female | 24.43 | 5.51 | 10.29–38.99 |
| | Both sexes | 25.28 | 8.35 | 10.29–64.95 |
| Erythrocyte nucleocytoplasmic ratio (NA/EA) | Male | 0.17 | 0.07 | 0.08–0.47 |
| | Female | 0.15 | 0.04 | 0.06–0.26 |
| | Both sexes | 0.16 | 0.06 | 0.06–0.47 |
| Immature erythrocyte diameter (μm) | Male | 14.80 | 2.36 | 9.64–20.28 |
| | Female | 15.03 | 2.04 | 9.64–20.39 |
| | Both sexes | 14.91 | 2.20 | 9.64–20.39 |
| Immature erythrocyte area (IEA; μm$^2$) | Male | 176.28 | 54.07 | 72.94–322.91 |
| | Female | 180.69 | 48.15 | 72.94–326.58 |
| | Both sexes | 178.49 | 51.16 | 72.94–326.58 |
| Immature erythrocyte nuclear diameter (μm) | Male | 7.29 | 1.26 | 4.50–9.97 |
| | Female | 7.33 | 1.27 | 4.50–9.97 |
| | Both sexes | 7.31 | 1.26 | 4.50–9.97 |
| Immature erythrocyte nuclear area (NIEA; μm$^2$) | Male | 43.03 | 14.71 | 15.89–78.03 |
| | Female | 43.45 | 14.91 | 15.89–78.03 |
| | Both sexes | 43.24 | 14.78 | 15.89–78.03 |
| Immature erythrocyte nucleocytoplasmic ratio* (NIEA/IEA) | Male | 0.27 | 0.14 | 0.07–1.01 |
| | Female | 0.26 | 0.13 | 0.06–1.04 |
| | Both sexes | 0.27 | 0.14 | 0.06–1.04 |
| Thrombocyte length (μm) | Male | 13.02 | 3.54 | 6.30–20.14 |
| | Female | 14.85 | 2.36 | 7.68–19.55 |
| | Both sexes | 13.93 | 3.14 | 6.30–20.14 |
| Thrombocyte width (μm) | Male | 6.57 | 1.25 | 4.20–9.92 |
| | Female | 7.54 | 1.18 | 4.50–10.74 |
| | Both sexes | 7.05 | 1.31 | 4.20–10.74 |

[a] Data are derived from 5 males, 5 females or both (10 males and females) frogs.
* Significant difference in the mean nucleocytoplasmic ratios between mature erythrocytes (NA/EA) and immature erythrocytes (NIEA/IEA), Student's *t*-test ($P \leq 0.05$).

elliptical nucleus. The clear cytoplasm was scarce and stained light blue or colorless (Figure 1B). The cells were sometimes found grouped together on the blood smears. Leukocytes were separated into agranulocytes and granulocytes and then further classified into two and three cell types, respectively, based on the morphological characteristics of the Giemsa stained blood smears. Agranulocytes were classified into lymphocytes and monocytes. Lymphocytes were round or slightly elliptical shaped cells that contained small amount of cytoplasm. The nucleus was compact and dark stained, positioned centrally in the cell and covered by a light-blue stained cytoplasm (Figure 1C). Monocytes had a relatively higher cytoplasmic to nuclear ratio than large lymphocytes and contained a round, kidney- or horseshoe-shaped nucleus adjacent to the cell edge. The nucleus had less intensely

**Table 3** Morphometric data of leukocytes (cell diameter) of *H. rugulosus* collected from Nan Province, Thailand during July to August 2014.

| Diameters | Sex[a] | Mean | SD | Range |
|---|---|---|---|---|
| Lymphocytes (μm) | Male | 11.02 | 2.87 | 5.94–18.96 |
| | Female | 11.00 | 2.51 | 5.18–18.74 |
| | Both sexes | 11.01 | 2.69 | 5.18–18.96 |
| Monocytes (μm) | Male | 12.46 | 2.45 | 6.66–18.31 |
| | Female | 11.63 | 2.28 | 8.03–19.73 |
| | Both sexes | 12.04 | 2.40 | 6.66–19.73 |
| Neutrophils (μm) | Male | 12.84 | 2.08 | 9.52–18.11 |
| | Female | 12.23 | 2.07 | 7.83–15.79 |
| | Both sexes | 12.58 | 2.08 | 7.83–18.11 |
| Basophils (μm) | Male | 13.42 | 2.11 | 10.06–17.49 |
| | Female | 13.73 | 2.26 | 10.08–17.49 |
| | Both sexes | 13.60 | 2.17 | 10.06–17.49 |
| Eosinophils (μm) | Male | 12.92 | 3.30 | 6.90–20.15 |
| | Female | 11.75 | 2.42 | 7.33–18.48 |
| | Both sexes | 12.33 | 2.95 | 6.90–20.15 |

[a] Data are derived from 5 males, 5 females or both (10 males and females) frogs.

stained chromatin than that of the lymphocytes (Figure 1D).

Granulocytes were classified into three cell types (neutrophils, eosinophils and basophils) based on the characteristics of their nuclei and cytoplasmic granules. Neutrophils were round cells, marginally larger (1.04-fold) than monocytes and had a multiple-lobed nucleus, like in humans. However, some cells contained nuclei that looked U-shaped. The cytoplasm contained fine granules and stained light purple (Figure 1E). Eosinophils were sized in between neutrophils and monocytes with a less segmented nucleus, and relatively large cytoplasmic granules of a round to elliptical shape that stained red brown (Figure 1F). Basophils were fairly large cells, slightly (1.03-fold) larger than large lymphocytes, and were characterized by the presence of round highly basophilic (dark blue) granules of various sizes in the cytoplasm. The inconspicuous round nucleus was positioned in the center of the cell (Figure 1G).

## 4. Discussion

Data on the hematological parameters of amphibians in Asia is still limited. This study is the first report of the hematological parameters in *H. rugulosus* in Southeast Asia. The number of erythrocytes, as determined by hemocytometer counts, in wild-caught *H. rugulosus* was higher than that previously reported for other anurans, including that of a closely related species, *R. tigrina* (Singh, 1977a).

The presence of immature erythrocytes in the circulating blood can occur under normal (healthy) conditions in vertebrates, but if this reaches above the normal value it indicates stress-related condition in erythropoiesis (Briggs and Bain, 2012). In general, an abnormal increase in the number of immature erythrocytes in the circulating blood indicates a compensatory regenerative response due to anemia or loss of circulating erythrocytes (Allender and Fry, 2008). Because of the lack of baseline data of circulating immature erythrocyte levels in *H. rugulosus* it was not possible to determine if these are normal or abnormal levels of circulating immature erythrocytes, but the external appearance of the frogs were normal and healthy.

Sex-related differences in some blood parameters are known to exist in mammals and other vertebrates (Golemi *et al.*, 2013; Nemeth *et al.*, 2010; Xie *et al.*, 2013). From the blood smear examinations of *H. rugulosus*, the differential lymphocyte and neutrophil counts showed sex-related difference with higher values in males. However, very few researches have reported sex-related differences of these parameters previously in amphibians. A study in wild-caught Indian tree frog (*Polypedates maculatus*) reported significantly higher monocyte and eosinophil counts in males than in females (Mahapatra *et al.*, 2012), while the neutrophil/lymphocyte ratio was significantly higher in wild-caught female mole salamanders (*Ambystoma talpoideum*), which was attributed to reproductive stress (Davis and Maerz, 2008). Given that the sampling period used in this study was with the reproductive period of *H. rugulosus* this may have been the cause of the observed sex-related differences in the blood parameters detected in this study.

From the differential leukocyte counts, agranulocytes were found to comprise a seven-fold higher proportion of the leukocytes than granulocytes (88% and 12% of all

leukocytes, respectively). The most abundant leukocytes in *H. rugulosus* peripheral blood were lymphocytes, which is similar to previous reports in other frog species (Arikan and Cicek, 2014; Cabagna *et al.*, 2005; Cathers *et al.*, 1997; Das and Mahapatra, 2012; Singh, 1977b).

The PCV of these *H. rugulosus* also showed a significant sex-related difference with a higher PCV value in males. The PCV value has been reported to indicate the erythrocyte mass in amphibians (Allender and Fry, 2008). However, the mean PCV value of *H. rugulosus* regardless of the gender (28%) was similar to those reported in other frogs (Cabagna *et al.*, 2005; Cathers *et al.*, 1997; Donmez *et al.*, 2009; Gul *et al.*, 2011; Mahapatra *et al.*, 2012; Sinha, 1983; Wojtaszek and Adamowicz, 2003), but lower than those of salamander (Solis *et al.*, 2007). The difference in PCV values among amphibians is believed to depend on differences in sex, season, habitat and natural history of each species.

The morphology of blood cells in amphibians is similar in terms of their general characteristics, such as cell shape, nuclear shape and granules in cytoplasm. But some specific characteristics may vary among species, especially the size of each cell type. In this study, the erythrocyte size, determined as dimensions and area, fell within the ranges reported for other anurans (Arikan and Cicek, 2010). The significant difference between the nucleocytoplasmic ratios of circulating mature and immature erythrocytes in *H. rugulosus* confirmed the identification criteria of immature cells, where a higher nucleocytoplasmic ratio indicate less mature cells. The mean nucleocytoplasmic ratio of circulating erythrocytes in these *H. rugulosus* was in the range reported in other anurans (Arikan and Cicek, 2014). However, the mean length and width of thrombocytes in these *H. rugulosus* suggested that this cell type is more ellipsoid than other anurans, while leukocytes also showed a difference in size (diameter) of each leukocyte type compared with that in other anuran species (Arikan and Cicek, 2010). The largest circulating leukocytes in *H. rugulosus* were basophils and the smallest were lymphocytes.

## 5. Conclusion

Based on their morphological characteristics, cells in the peripheral blood of *H. rugulosus* were classified into mature and immature erythrocytes, leukocytes (lymphocytes, monocytes, neutrophils, basophils and eosinophils) and thrombocytes. The presence of immature erythrocytes, even though frequently reported in vertebrates, is interesting since an increase in their circulating level can indicate a disturbance of the erythron. Thus, the data obtained in this study will be useful in assessing abnormality in the erythron of *H. rugulosus* in the future. Moreover, these results revealed that the PCV and the differential lymphocyte and neutrophil counts of male frogs were significantly higher than those of the females. However, the size of each blood cell type was not significantly different between sexes of *H. rugulosus*. The hematological parameters presented in this study are the first report for *H. rugulosus* and so represent the crucial baseline data for wild *H. rugulosus* in Thailand that can be expanded upon to use for monitoring the health status of this anuran in the future.

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